than 60 (Shulman *et al.*, 2006). With aging, acquired skewing occurs in normal females and is present in 38% of women over the age of 60 years (Busque *et al.*, 1996), whereas truly random lyonization is rare (El Kassar *et al.*, 1998). Whether acquired skewing is a consequence of stem cell depletion, true clonal tumor expansion, growth advantage conferred by parental-specific X-chromosomes, or other unknown mechanisms has not been elucidated yet. Unfortunately, the authors do not address the putative influence of skewing on the outcome of their study, although the broad range of variation conferred by this mechanism might provide a better explanation for their data. A further disadvantage of X-chromosome inactivation studies of tumors is that they are only informative when interpreted in the context of the clonal composition of the surrounding normal tissue and correlated with an adequate sample number of normal control tissue from age-matched individuals (Novelli *et al.*, 2003). We feel that the latter aspect was also insufficiently addressed by Shulman *et al.* because they did not incorporate age-matched controls for lyonization. Another important factor, often ignored in such studies, is the distribution of X-inactivated cells in tissues. Taking into consideration that lyonization occurs early in development, many of the progeny of a single embryonic stem cell are grouped together in the adult, forming patches. As polyclonality can only be demonstrated at the borders of X-inactivation patches, not only the patch size is crucial in determining the chance of demonstrating polyclonality but also the number of tumors that need to be examined to exclude polyclonality (Novelli *et al.*, 2003) – one more aspect not considered by the authors.

In conclusion, we believe that the authors did not deliver sufficient evidence to support their theory that anatomically and temporally distinct BCCs can originate from one single tumor cell clone.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Response to van Steensel and Frank

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TO THE EDITOR

To the comments and concerns raised by Drs van Steensel and Frank (*Journal of Investigative Dermatology* advance online publication, 27 July 2006, doi: 10.1038/sj.jid.5700471), regarding the main conclusion contained in our manuscript ''Monoclonal origin of anatomically distinct basal cell carcinomas'' (Shulman *et al.*, 2006), we offer the following responses:

1. It was never claimed anywhere by us in the manuscript that the clone precursor cell of anatomically distinct basal-cell carcinoma (BCC) represents a metastatic process. If we use the classical example of another disorder, namely McCune–Albright syndrome (OMIM# 174800), it is clear that in this syndrome, there is a post-zygotic mutation in the Gs alpha gene – so-called *gsp* mutation – that is contained in all affected tissues (café au lait spots included), and has been acquired during embryogenesis (Weinstein *et al.*, 1991). It is evident that the McCune–Albright syndrome is not

Abbreviations: BCC, basal-cell carcinoma; LOH, loss of heterozygosity; PTCH, patched

a cancerous process, and the existence of a similar mutation in all affected tissues is indicative of the monoclonal and common precursor cell in this disease entity, rather than a metastatic spread. Similarly, it is totally plausible that a similar process that involves a post-zygotic mutation in a tumor suppressor gene (patched (PTCH) gene or other unidentified gene) could underlie and account for our data.

2. We are surprised to read Drs van Steensel and Frank's comment and critique that loss of heterozygosity (LOH) determination and X-chromosome inactivation patterns are invalid technologies and approaches to assessing tumor monoclonal origin. There are many articles that have assessed clonality using the combination of both techniques and applied for both benign and malignant tumors (Arnold *et al*., 1988; Friedman *et al*., 1989). In a quote from a recent review of the clonal origin of benign pituitary tumors, Clayton and Farrell (2004) note, ''Allelotype analysis and X-chromosome inactivation analysis in women enables the assessment of tissue clonality''. Similarly, Diaz-Cano *et al*. (2001), who reviewed the techniques for assessing clonality state, ''LOH analyses identify clonal expansions of a tumor cell population, and point to monoclonal proliferation when multiple and consistent LOH are demonstrated. Based on the methylation-related inactivation of one X chromosome in female subjects, X-linked markers (e.g., androgen receptor gene) will provide clonality information using LOH analyses after DNA digestion with methylation-sensitive restriction endonucleases''.
3. We agree that analysis of patients for PTCH gene mutations would strengthen the data. However, if the tumor suppressor gene targeted by the LOH is an unidentified gene, even the lack of PTCH gene germline mutations would not preclude or override our data.
4. We chose the markers to test LOH by applying the ones that were successful in previous allelotyping studies of BCCs (Iwata *et al*., 2004). If these markers are outside of the region containing the PTCH gene, they might indicate that a tumor suppressor gene, distinct from PTCH, that resides within the same chromosomal region is targeted for inactivation by the LOH. Hence, even not finding recurrent somatic mutations in the distinct tumor samples would not negate our findings. We would welcome, in a collaborative manner and spirit, any assistance from Drs van Steensel and Frank in sequencing the tumor samples for PTCH gene mutations.
5. We are surprised by the comments regarding the meiotic recombination and its putative effect on LOH. The LINE element is present in the tumor tissue as well as in germline DNA. As LOH by definition is a somatic event, why did the recombination occurred preferentially and exclusively in the tumor tissue? In addition, we are well aware of other genes and loci involved in the pathogenesis of BCC. We fail to see the relevance of this information to the LOH data, or any other aspect of our study. The most important and crucial finding in our study is the fact that in the *same patient*, anatomically distinct tumors (as many as four in one case) always showed LOH of the *same allele*. The odds that this phenomenon represents a chance occurrence or a mere coincidence is statistically negligible, despite the beliefs echoed in the comment by Drs van Steensel and Frank.
6. Random extreme lyonization is indeed rare. Even though acquired X-inactivation pattern is more prevalent in aging women, it seems unlikely to explain the data: we used normal surrounding tissue as normal control tissue. Acquired skewed X inactivation does not preferentially occur in tumor tissue. Thus, the use of ''adequate sample of normal surrounding tissue'' requested by Drs Van Steensel and Frank is met. In essence, this use also means that requiring similar analysis of age-matched controls is redundant and superfluous. Furthermore, only one tumor in the analysis of X-chromosome inactivation was the determining factor in evaluating clonality. In all others, the evaluation and assignment of clonality was primarily based on LOH and only supported by X-chromosome inactivation patterns.
7. In conclusion, without invoking our own beliefs, we employed time-honored, well-accepted, and practiced techniques to assess monoclonal origin of BCC and provide a plausible, novel insight into the clonal origin of anatomically distinct BCC.

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